PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

REC'D 13 JAN 2006
WIPO PCT

Applicant's or agent's file reference BM002PCT	FOR FURTHER ACTION See Form PCT/IPEA/416	
International application No. PCT/JP2004/016717	International filing date *(day/month/year)* 04.11.2004	Priority date *(day/month/year)* 04.11.2003
International Patent Classification (IPC) or national classification and IPC C12N5/08, A61K35/12, A61P19/00		
Applicant BIOMASTER, INC. et al.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 7 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 16 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☒	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☒	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
05.10.2005	16.01.2006
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer Teyssier, B Telephone No. +31 70 340-2062

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/JP2004/016717

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-6, 9-24, 26-31, 33-60, 62-113	as published
7, 8, 25, 32, 61	filed with telefax on 05.10.2005

Claims, Numbers

49-68	as published
1-48, 69-72	filed with telefax on 05.10.2005

Claims, Pages

122-125	as published
114-120, 121/1, 121/2, 126, 127	filed with telefax on 05.10.2005

Drawings, Sheets

1-26	as published

☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☒ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☒ the claims, Nos. 16, 17, 25
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 47, 67, 70, (IA)

because:

☒ the said international application, or the said claims Nos. 47, 67, 70 (IA) relate to the following subject matter which does not require an international preliminary examination (specify):

see separate sheet

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos.

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form ☐ has not been furnished

☐ does not comply with the standard

the computer readable form ☐ has not been furnished

☐ does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/JP2004/016717

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-15, 18-24, 26-31, 40-54, 67-72
	No:	Claims	32-39, 55-66
Inventive step (IS)	Yes:	Claims	1-15, 18-24, 26-31, 40-54, 67-72
	No:	Claims	32-39, 55-56
Industrial applicability (IA)	Yes:	Claims	1-15, 18-24, 26-46, 48-66, 68, 69, 71, 72
	No:	Claims	-

2. Citations and explanations (Rule 70.7):

see separate sheet

Box No. VI Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

0 The following numbering is used:
D1 WO 01/62901 A (Artecell Sciences, Inc.) 30 August 2001
D2 WO 00/53795 A (University of Pittsburg; University of California) 14 September 2000
D2 WO 03/022988 A (University of California) 20 March 2003
D4 Zuk P A et al., *Tissue Engineering* April 2001, 7(2), 211-228
D5 Gimble J M & Guilak F, *Cytotherapy* 2005, 5(5), 362-369
D6 Gronthos S et al., *Bone* February 2001, 28(2), 174-181
D7 WO 2005/035738 A (Biomaster, Inc.) 21 April 2005

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

3.1 Claims 47, 67 and 70 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(I) PCT).

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

5.1 *Methods and systems for preparing stem cells, derived uses (claims 1-31, 40-54, 67-72)*

D1 teaches at p. 11-12 the preparation of adipose-derived stromal cells; adipose tissue, typically obtained by liposuction from a human subject, is treated with collagenase, filtered and subjected to differential centrifugation directly in media or over a Ficoll or Percoll or particulate gradient (p. 12, line 8-9). D2 and D3 teach the preparation of adipose-derived stromal cells by treating a liposuction aspirate with collagenase, centrifugation at 260 g, suspension in an erythrocyte-lysing buffer and centrifugation at 250 g (D2, ex. 1; D3, ex. 1, 9, 10). D3 and D4 teach the preparation of adipose-derived stromal cells by treating a liposuction aspirate with collagenase and pelleting the cells by centrifugation at 1200 g (D3, ex. 8; D4, p. 212-213). No method for preparing adipose-derived stem cells without collagenase treatment has been described or suggested in the prior art, thus the subject-matter of claims 1-15, 18-24,and 26-31 is novel and an inventive step can be acknowledged (Article 33(2,3) PCT) and it follows therefrom that the subject-matter of the further method claims 40-54 and 67-72 is also novel and inventive. While the examples of the present application do not provide a direct comparison of the adipose-derived stem cells obtained with or without collagenase treatment (comparative example 14

vs. representative example 2) which would allow this Authority to appreciate any improved effect obtained by the method of the application (e.g. yield, viability), an inventive step can be acknowledged for the sole motive of streamlining the preparation process by removing a step of enzymatic treatment.

5.2 *Stem cells, differentiated cells and compositions (claims 32-39, 55-66)*

Documents D1-D5, the latter being a short review on the topic, all disclose adipose-derived stromal cells and their applications as multi- or pluripotent stem cells for *in vitro* or *in vivo* differentiation and for therapy, therefore the subject-matter of *product* claims 32-39 and 55-66 is not new in view of any of D1-D5, regardless of the process by which these cells are obtained (Article 33(2) PCT; PCT Guidelines A5.26[1]). It is not necessary to consider the set of markers displayed (or not) by the cells, as this is an intrinsic property of adipose-derived stromal cells. It may also be observed that, while some discrepancies have been noted with respect to markers (see D5, § bridging p. 363-364), these are not regarded as significant and that an inventive step for a possibly new subpopulation of adipose-derived stromal cells could only be acknowledged in view of experimental data associating the particular phenotype of the subpopulation with a specific and useful property.

5.3 For the assessment of the present claims 45-47 and 57-72 on the question whether they are industrially applicable, no unified criteria exist in the PCT contracting states. The patentability can also be dependent upon the formulation of the claims.

Re Item VI

Certain documents cited

6.1 D7, filed on 10 March 2004 and claiming priorities of 7 October 2003 and 16 February 2004, discloses the process(es) of the invention at pages 41-42 and example 2. This document, published on 21 April 2005 and not belonging to the prior art under Rule 64.1 PCT, may nevertheless become relevant during national or regional prosecution of the application (Rule 64.3 PCT).

Re Item VIII

Certain observations on the international application

8.1 The International Preliminary Examining Authority welcomes the amendments introduced in order to clarify that the purpose of the application is the purification of adipose-derived stromal cells without collagenase treatment. Nevertheless, further editorial amendments may be required to distinguish further the subject-matter of the claimed invention from prior art, or representative examples of the

invention from comparative examples (e.g. example 3 vs. example 15).

8.2 Despite the amended wording of claim 70, the subject-matter of said claim still appears to fall under Rule 67.1(iv) PCT because the introduction of living cells into a subject's body is such a major intervention that it can hardly be reduced to a matter of "cosmetic", and the administration may well require the use of surgery, which falls under Rule 67.1(iv) PCT regardless whether the method is therapeutic or cosmetic surgery.

13. The method according to Item 1, further comprising the step of removing blood cells.

14. A method for preparing a stem cell comprising:
A) obtaining material from liposuction; and
B) subjecting the material from liposuction to centrifugation to obtain a cell fraction without isolation of fat tissue.

15. The method according to Item 14, further comprising the step of subjecting the material to a condition where at least a portion of cells are separated from the material.

~~16. The method according to Item 15, wherein the condition is for degradation of extracellular matrices.~~

~~17. The method according to Item 15, said degradation of extracellular matrices is achieved by a collagenase.~~

18. The method according to Item 14, further comprising the step of removing supernatant in step B).

19. The method according to Item 14, further comprising the step of filtering the material from the step B).

20. The method according to Item 14, further comprising the step of removing blood cells.

21. The method according to Item 14 wherein the step of removing blood cells comprises adding a component of degrading blood cells.

22. A method for preparing a stem cell comprising:

i) obtaining material from liposuction;

ii) subjecting the material to a condition where at least a portion of cells are separated from the material, without isolation of fat tissue;

iii) subjecting the material to centrifugation;

iv) adding a component degrading blood cells to the material and agitating the material;

v) subjecting the material to centrifugation to obtain a pellet; and

vi) aspirating supernatant of the material from the pellet.

23. The method according to Item 22, wherein the step of subjecting the material to said condition comprises maintaining an aspirate from the liposuction.

24. The method according to Item 22, wherein said material from liposuction comprises an aspirate from liposuction and fat.

~~25. The method according to Item 22, wherein said condition in said step ii) comprises adding a collagenase.~~

26. The method according to Item 22, wherein the centrifugation in said step iii) is conducted at 400-1200 x g.

27. The method according to Item 22, wherein said component degrading blood cells comprises ammonium chloride and potassium bicarbonate.

cell, which has monopotency, multipotency, or totipotency. Stem cells can be differentiated in response to specific stimuli. Typically, stem cells can regenerate an injured tissue. Stem cells used herein may be, but are not limited to, ~~embryonic stem (ES) cells,~~ tissue stem cells (also called tissular stem cell, tissue-specific stem cell, or somatic stem cell), or other precursor cells. A stem cell may be an artificially produced cell (e.g., fusion cells, reprogrammed cells, or the like used herein) as long as it can have the above-described abilities. Embryonic stem cells are pluripotent stem cells derived from early embryos. An embryonic stem cell was first established in 1981, which has been applied to production of knockout mice since 1989. In 1998, a human embryonic stem cell was established, which is currently becoming available for regenerative medicine. Tissue stem cells have a relatively limited level of differentiation unlike embryonic stem cells. Tissue stem cells are present in tissues and have an undifferentiated intracellular structure. Tissue stem cells have a higher nucleus/cytoplasm ratio and have few intracellular organelles. Most tissue stem cells have pluripotency, a long cell cycle, and proliferative ability beyond the life of the individual. As used herein, stem cells may be ~~preferably embryonic stem cells, though~~ tissue stem cells ~~may also be~~ employed depending on the circumstance.

Tissue stem cells are separated into categories based on the sites from which the cells are derived, such as the dermal system, the digestive system, the bone marrow system, the nervous system, and

function and/or form in a multicellular organism. "Tissue" is typically an aggregate of cells of the same origin, but may be an aggregate of cells of different origins as long as the cells have the same function and/or form. Therefore, when stem cells of the present invention are used to regenerate tissue, the tissue may be composed of an aggregate of cells of two or more different origins. Typically, a tissue constitutes a part of an organ. Animal tissues are separated into epithelial tissue, connective tissue, muscular tissue, nervous tissue, and the like, on a morphological, functional, or developmental basis. ~~Plant tissues are roughly separated into meristematic tissue and permanent tissue according to the development stage of the cells constituting the tissue.~~ Alternatively, tissues may be separated into single tissues and composite tissues according to the type of cells constituting the tissue. Thus, tissues are separated into various categories. Any tissue may be herein intended as a target to be treated.

Any organ may be targeted by the present invention. A tissue or cell targeted by the present invention may be derived from any organ. As used herein, the term "organ" refers to a morphologically independent structure localized at a particular portion of an individual organism in which a certain function is performed. In multicellular organisms (e.g., animals, plants), an organ consists of several tissues spatially arranged in a particular manner, each tissue being composed of a number of cells. An example of such an organ includes an organ relating to the vascular system. In one embodiment, organs targeted by the present

The material from liposuction used in the present invention usually includes an aspirate from liposuction and fat, however, it was found that when treated according to the preset invention, the material contains many more stem cells than that found in an aspirate.

~~Preferably, said condition in said step ii) comprises adding a collagenase.~~

Preferably, the present method may further comprise the step of subjecting the material to said condition comprises maintaining an aspirate from the liposuction.

Preferably, the material from liposuction used in the present invention, may further comprises an aspirate from liposuction and fat.

In another embodiment, the centrifugation in said step iii) is conducted at 400-1200 x g. Usually 400 x g or 800 x g is used.

In another embodiment, said component degrading blood cells comprises ammonium chloride and potassium bicarbonate.

In another embodiment, said ammonium chloride is comprised in the component at 100 mM to 200 mM, preferably at about 155mM. In another embodiment, said potassium bicarbonate is comprised in the component at 5 mM to 20 mM, preferably about 10mM. Preferably, the combination of the two is advantageously used.

CLAIMS

What is claimed is:

1. (Amended) A method for preparing a stem cell, without collagenase treatment, comprising:

A) obtaining an aspirate from liposuction;

B) subjecting the aspirate from liposuction to centrifugation to obtain a cell fraction

C) subjecting the cell fraction to centrifugation by specific gravity; and

D) collecting a cell layer with lower specific gravity than that of erythrocytes.

2. The method according to Claim 1, wherein said aspirate from liposuction is prepared using saline or Ringer's solution.

3. The method according to Claim 1, wherein said centrifugation is conducted at a speed of a range equal to or less than 800 x g.

4. The method according to Claim 1, wherein said centrifugation is conducted at a speed of a range equal to or less than 400 x g.

5. The method according to Claim 1, wherein said centrifugation by specific gravity is conducted at a speed of a range between 370 x g and 1,100 x g.

6. The method according to Claim 1, wherein said centrifugation by specific gravity is conducted using medium which as a specific gravity of 1.076 to 1.078

g/ml at 20 degree Celsius.

7. The method according to Claim 1, wherein the medium of said centrifugation by specific gravity is selected from the group consisting of Ficoll, Percoll and sucrose.

8. The method according to Claim 7, wherein the medium of said centrifugation by specific gravity is Ficoll.

9. The method according to Claim 1, wherein the specific gravity of the collected cell layer is at a range of between 1.050 and 1.075.

10. The method according to Claim 1, wherein the collection of said cell layer is conducted using a pipette.

11. The method according to Claim 1, further comprising the step of culturing said cell layer in a medium containing components selected from the group consisting of DMEM, M199, MEM, HBSS, Ham's F12, BME, RPMI1640, MCDB104, MCDB153(KGM) and a mixture thereof.

12. The method according to Claim 1, wherein the centrifugation by specific gravity comprises density gradient centrifugation.

13. The method according to Claim 1, further comprising the step of removing blood cells.

14.(Amended) A method for preparing a stem cell,

without collagenase treatment, comprising:

A) obtaining material from liposuction; and

B) subjecting the material from liposuction to centrifugation to obtain a cell fraction without isolation of fat tissue.

15. The method according to Claim 14, further comprising the step of subjecting the material to a condition where at least a portion of cells are separated from the material.

(Cancelled) [~~16. The Method according to Claim 15, wherein the condition is for degradation of extracellular matrices.~~]

(Cancelled) [~~17. The method according to Claim 15, said degradation of extracellular matrices is achieved by a collagenase.~~]

18. The method according to Claim 14, further comprising the step of removing supernatant in step B).

19. The method according to Claim 14, further comprising the step of filtering the material from the step B).

20. The method according to Claim 14, further comprising the step of removing blood cells.

21. The method according to Claim 14 wherein the step of removing blood cells comprises adding a component of degrading blood cells.

22.(Amended) A method for preparing a stem cell, without collagenase treatment, comprising:

i) obtaining material from liposuction;

ii) subjecting the material to a condition where at least a portion of cells are separated from the material, without isolation of fat tissue;

iii) subjecting the material to centrifugation;

iv) adding a component degrading blood cells to the material and agitating the material;

v) subjecting the material to centrifugation to obtain a pellet; and

vi) aspirating supernatant of the material from the pellet.

23. The method according to Claim 22, wherein the step of subjecting the material to said condition comprises maintaining an aspirate from the liposuction.

24. The method according to Claim 22, wherein said material from liposuction comprises an aspirate from liposuction and fat.

(Cancelled)[~~25. The method according to Claim 22, wherein said condition in said step ii) comprises adding a collagenase.~~]

26. The method according to Claim 22, wherein the centrifugation in said step iii) is conducted at 400-1200 × g.

27. The method according to Claim 22, wherein said component degrading blood cells comprises ammonium chloride and potassium bicarbonate.

28.(Amended) The method according to Claim 27, wherein said ammonium chloride is comprised in the component at 155mM.

29. The method according to Claim 27, wherein said potassium bicarbonate is comprised in the component at 10mM.

30. The method according to Claim 22, wherein said centrifugation in said step v) is conducted at 400-1200 x g.

31. The method according to Claim 22, wherein said pellet contains a stem cell.

32. A stem cell prepared by the method according to any of Claims 1-31.

33. The stem cell according to Claim 32, which expresses at least one protein selected from the group consisting of CD13, CD29, CD34, CD36, CD44, CD49d, CD54, CD58, CD71, CD73, CD90, CD105, CD106, CD151 and SH3.

34. The stem cell according to Claim 33, which expresses CD13, CD29, CD34, CD36, CD44, CD49d, CD54, CD58, CD71, CD73, CD90, CD105, CD106, CD151 and SH3.

35. The stem cell according to Claim 33, further expressing at least one protein selected from the group consisting of CD31, CD45, CD117 and CD146.

36. The stem cell according to Claim 32, which does not express CD56.

37. The stem cell according to Claim 32, which does not express at least one protein selected from the group consisting of CD3, CD4, CD14, CD15, CD16, CD19, CD33, CD38, CD56, CD61, CD62e, CD62p, CD69, CD104, CD135 and CD144.

38. The stem cell according to Claim 37, which does not express CD3, CD4, CD14, CD15, CD16, CD19, CD33, CD38, CD56, CD61, CD62e, CD62p, CD69, CD104, CD135 and CD144.

39. The stem cell according to Claim 32, which expresses CD49d and does not express CD56.

40. (Amended) A system for preparing a stem cell, without collagenase treatment, comprising:

A) means for obtaining an aspirate from liposuction;

B) means for subjecting the aspirate from liposuction to centrifugation to obtain a cell fraction; and

C) means for subjecting the cell fraction to centrifugation by specific gravity.

41. The system according to Claim 40, wherein the system further comprises:

D) means for collecting a cell layer with lower specific gravity than that of erythrocytes.

42.(Amended) A system for preparing a stem cell,

Without collagenase treatment, comprising:

A) means for obtaining material from liposuction; and

B) means for subjecting the material from liposuction to centrifugation to obtain a cell fraction without isolation of fat tissue.

43.(Amended) A system for preparing a stem cell, without collagenase treatment, comprising:

i) means for obtaining material from liposuction;

ii) means for subjecting the material to a condition where at least a portion of cells are separated from the material, without isolation of fat tissue;

iii) means for subjecting the material to centrifugation;

iv) a component degrading blood cells to the material and agitating the material;

v) means for subjecting the material to centrifugation to obtain a pellet; and

vi) means for aspirating supernatant of the material from the pellet.

44.(Amended) A method for obtaining an explant, without collagenase treatment, comprising:

A) obtaining an aspirate from liposuction;

B) subjecting the aspirate from liposuction to centrifugation to obtain a cell fraction;

C) subjecting the cell fraction to centrifugation by specific gravity;

D) collecting a cell layer with lower specific gravity than that of erythrocytes;

E) culturing the collected cell layer to obtain an

explant.

45.(Amended) A method for preparing a tissue transplant, without collagenase treatment, comprising:

A) obtaining an aspirate from liposuction;

B) subjecting the aspirate from liposuction to centrifugation to obtain a cell fraction; and

C) culturing the collected cell layer to obtain a tissue transplant.

46.(Amended) A method for preparing tissue transplant, without collagenase treatment, comprising:

A) obtaining an aspirate from liposuction;

B) subjecting the aspirate from liposuction to centrifugation to obtain a cell fraction;

C) subjecting the cell fraction to centrifugation by specific gravity;

D) collecting a cell layer with lower specific gravity than that of erythrocytes;

E) culturing the collected cell layer to obtain a tissue transplant.

47.(Amended) A method for transplanting a tissue transplant, without collagenase treatment, comprising:

A) obtaining an aspirate from liposuction;

B) subjecting the aspirate from liposuction to centrifugation to obtain a cell fraction;

C) subjecting the cell fraction to centrifugation by specific gravity;

D) collecting a cell layer with lower specific gravity than that of erythrocytes;

E) culturing the collected cell layer to obtain a tissue transplant; and

F) transplanting the tissue transplant.

48. (Amended) Use of an aspirate of liposuction in preparing stem cells, without collagenase treatment.

49. A method for preparing cells selected from the group consisting vascular endothelial precursor cells, adipocytes, cartilage cells, bone cells and muscle cells comprising the step of culturing a stem cell

disease, a disorder or an abnormal condition attributed to the deficiency of a differentiated cell, comprising:

a) a stem cell obtained according to any one of Claims 1-31;

b) a differentiated cell corresponding to a desired site; and

c) a pharmaceutically acceptable carrier.

69. Use of a mixture of: a) a stem cell obtained according to any one of Claims 1-31; and b) a differentiated cell corresponding to a desired site, for preparation of a medicament for treatment or prevention of a disease, a disorder or an abnormal condition attributed to the deficiency of a differentiated cell.

70. (Amended) A method for [~~treatment or~~] improvement of a cosmetic condition, comprising the steps of:

A) providing a composition comprising:

a) a stem cell obtained according to any one of Claims 1-26; and

b) a differentiated cell corresponding to a desired site; and

B) administering the composition to a subject.

71. (Amended) A [~~medicament~~] composition for [~~treatment or~~] improvement of a cosmetic condition, comprising:

a) a stem cell obtained according to any one of Claims 1-31;

b) a differentiated cell corresponding to a desired site; and

c) a pharmaceutically acceptable carrier.

72.(Amended) Use of a mixture of: a) a stem cell obtained according to any one of Claims 1-31; and b) a differentiated cell corresponding to a desired site, for preparation of a [~~medicament~~] composition for [~~treatment or~~] improvement of a cosmetic condition.